NOTES ON NAGANA AND ON SOME HAEMATOZOA OBSERVED DURING MY TRAVELS.

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I. Nagana.

Several observations on the disease caused by the *Trypanosoma brucei*, which were made after the publication of the paper by Kanthack, myself and Blandford[1], have not yet been published, and since the points have not attracted the attention of other writers, it seems worth while to place them on record.

Longevity of guinea-pigs suffering from Nagana.

It was stated in the above cited paper that the longest period of survival, which till then had been observed in guinea-pigs, was 183 days. A still smaller maximum was observed by Laveran and Mesnil[2], namely only 61 days.

When the virus was first received from South Africa, the experiments with guinea-pigs at first led us to believe that this animal was refractory. The guinea-pigs which had been inoculated and brought to England survived and did not show any signs of infection. Similar failure occurred with the earlier inoculations, which were performed by us with blood from the successfully infected dog which was delivered to us[3]. Further experiment however showed that the guinea-pig is not refractory, and many observers have had no difficulty in maintaining the strain by means of this animal.

The following observations are of interest, as showing that guinea-pigs may possess a considerable degree of resistance to the trypanosome :—

Two guinea-pigs (" A " and " B ") were inoculated with blood of

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[1] *Proc. Roy. Soc.*, vol. XLIV. 1898, p. 100, and *Hyg. Rundschau*, vol. VIII. p. 1185.

[2] *Trypanosomes et Trypanosomiases*, Paris 1904.

[3] The syringe was used for all our earlier inoculations, later we used a surgical needle merely wetted with infective blood.

an infected horse on 3. v. 1897. Thereafter, though their blood was examined frequently, and though their temperatures showed occasional irregular rises, no trypanosomes were found in their blood. It may be presumed either that the infecting blood was at fault, or that they were resistant individuals. The sequel is somewhat in favour of the latter view. It may be noted here, that on the 43rd day after this first inoculation, rats were injected with the blood of each guinea-pig with negative result.

Guinea-pig " A " was reinoculated by the late Dr Kanthack on 10. ii. 98 with blood of a guinea-pig containing active trypanosomes. Its blood was examined at intervals, and from 6. iii. to 8. vi. 98 the presence of the trypanosome was noted. From that day onward trypanosomes were absent, so that on 24. xi. 98, this guinea-pig was again inoculated, the blood of an infected rat being used. The period covered was already more than nine months since the inoculation which had shown itself to be successful. Seventeen days after this third inoculation the blood was free from parasites, but by 24. ii. 99 the trypanosome had become common, and the animal seemed to be ill. Eventually it died on 3. iii. 99 or 99 days after the last inoculation. At the autopsy, the lymphatic glands were found to be exceptionally enlarged for a guinea-pig, the spleen was also much more enlarged than usual; in fact the appearances recalled those which obtain in the rat.

The further notes on guinea-pig " B " have unfortunately been lost.

Observations on another pair of guinea-pigs (" C " and " D ") may be cited; these were first inoculated by Dr Kanthack on 22. ii. 98 from an infected guinea-pig.

Guinea-pig " C " showed that it had been successfully infected, as trypanosomes were present in its blood 62 days later; eventually it died of the infection on the 102nd day.

Guinea-pig " D " is of greater interest. Trypanosomata were discovered in its blood 44 days after inoculation and again on 15. iv. and 25. iv. 98. Subsequent examination twice or thrice a month failed to reveal parasites. On 24. xi. 98 it was again inoculated, this time from a rat. Unfortunately on 20. ii. 99 it was given another inoculation. On 8. iii. 99 and again on 21. iii. its blood was crowded with trypanosomes. Death occurred three days later. Counting from the original infection, the animal survived no less than 13 months, whilst the survival after the first reinoculation was 120 days; it may be noted that this infection was done from the same rat that served in the case of "A." It would seem then that although individual guinea-pigs may show

a considerable resistance to infection with the nagana trypanosome, yet they are not solidly immunised by apparent recovery from one attack.

Relation of Birds to Nagana.

In our joint report the fact is recorded that we were unable to infect pigeons with nagana; this has been confirmed by other writers, for instance Laveran and Mesnil.

It seemed possible that other kinds of birds might have a different reaction, and having obtained a kestrel (*Falco tinnunculus*), I inoculated it in the breast with citrated rat's blood, which swarmed with Nagana trypanosomes. The injection, about 2 c.c. in bulk, was given on 10. XI. 98. Twenty days later (1. XII.) the breast was punctured, and two rats were inoculated with the blood that was obtained, although no recognisable trypanosomes were to be seen. One of these rats died on the 12th day with trypanosomes in its blood and great enlargement of the spleen; its death, however, was complicated with a septic inflammation of the pleura. The second rat died on the 13th day, apparently of uncomplicated nagana, its blood was teeming with the parasites and the chief enlargement of the lymphatic glands corresponded with the site of injection (right thigh).

On 11. XII. the blood of the kestrel was again examined both locally from the breast and from the foot, but nothing that could be recognised as trypanosomal was found. Two more rats were injected with blood from the breast on this day, 31 days after the original inoculation of the bird. Both these rats succumbed to nagana; one was killed on the 11th day with teeming masses of trypanosomes in its blood; the other died on the 16th day. In both the chief glandular enlargement was in the right inguinal region, corresponding to the inoculation in the thigh.

On 24. II. 99 two more rats were inoculated, but they died after 24 and 48 hours of septic mischief.

On 6. III. 99 two more rats were injected with blood from the bird's breast. One of these died 35 days later without signs of infection by nagana, the other survived.

A reinoculation of the bird was performed on 25. IV. subcutaneously in the breast with about 1 c.c. of highly infected citrated rat's blood. On the next day only bird's and rat's red blood corpuscles, haemoglobin crystals and leucocytes could be found in a sample removed for inspection. Fifteen days later a rat was inoculated with blood from the breast, the result was negative.

On 16. vi. 99 nearly the whole of the blood (about 5 c.c.) of a highly infected rat was injected subcutaneously as before into the breast of the kestrel. On the next day a few active trypanosomes together with clumped masses of dark crinkled rat red corpuscles, leucocytes and granular matter were seen in a sample removed. A rat inoculated with this sample died in ten days of nagana. The bird was again examined locally three days after the injection, no trace of haematozoa or of rat blood corpuscles could be found ; leucocytes however were abundant. Two rats inoculated died in 12 and 17 days of typical nagana.

On 30. vi. a rat was inoculated with negative results.

The kestrel remained in apparently good health. Owing to its fractious nature and the danger of injuring it, its temperature was not taken regularly, one day it was noted to be 41·7° C. ; this is in accordance with the well known high temperature of birds.

In order to control this experiment with the kestrel, a *pigeon* was treated in exactly the same manner and contemporaneously. The dose given amounted to nearly 10 c.c. of citrated rat's blood (there were 2,500,000 trypanosomes per cubic millimetre in the undiluted blood).

Rats were inoculated on each of the four following days ; only one of these died, death being within 48 hours apparently due to sepsis.

On 10. xi. 98 or 59 days after the first injection, a second inoculation of highly infective rat's blood was given. Rats were inoculated at various periods, all with negative result. The temperature record of the pigeon was rather higher than that of the kestrel, viz. 42·5° C.

Until actual observations have been made on birds of prey in nagana infested regions, it is only possible to say that they might conceivably be carriers of infection. Koch's observation that *Glossina* will feed on crocodiles is of interest in this connexion.

Remarks on the Pathogenic Action of *Trypanosoma brucei*.

If the course of the disease as it occurs in rats is compared with that which obtains in the rabbit, it is clear that the trypanosomal form of the parasite, as such in the circulating blood, has but a small share in the determination of illness. Thus a rat may have several hundred thousand trypanosomes in each cubic millimetre of its circulating blood, without showing grave signs of its rapidly approaching death. The same is true of guinea-pigs, for I have watched a buck which was most zealous in its attentions to a doe, a few minutes after counting 500,000 trypanosomes per cubic millimetre in its blood ; moreover the animal's

weight record showed a continuous steady gain in weight since the inoculation.

On the other hand, progressive wasting and local swellings make a rabbit into a truly melancholy sight long before death supervenes, yet even with the aid of the centrifuge, the presence of trypanosomes may not be discoverable, until within a few days of death. The following example may be cited: a rabbit (2650 grams) was infected with a needle wetted with infective blood, 28 days later the weight had fallen to 1440 grams, the nose and eyelids were much swollen and obstructed. It was killed and its tissues searched for the presence of trypanosomes. The blood revealed none, in fact the only place where the parasites were found was in the rib marrow, and there they were quite sparse. Bone marrow in other regions was examined with negative result. It might be thought that the presence of the parasite in so important a tissue as the rib marrow might be significant to explain the disease, were it not that the trypanosome occurs in great numbers in the similar marrow of the rat. It is, perhaps, not inapt to remark that in malaria, the mature or almost mature parasites do not assert their presence with the marked disturbance of health, which is caused by the shower of a young brood through the system.

In animals, especially when they are near unto death from nagana disease, the blood shows marked morphological changes; the red blood corpuscles are reduced to about half their proper number, the leucocytes may be considerably increased, and nucleated red corpuscles are to be found. In my experience nucleated red corpuscles appear more abundantly in the rat and mouse than in other animals. Thus on one occasion the blood count of a rat, which was moribund, gave the high figure, 4,500 nucleated red corpuscles and 28,500 leucocytes per cubic millimetre. At this late stage of the disease the red corpuscles do not run well into rouleaux but tend to aggregate into small clumps. This clumping of the red corpuscles is evidently due to an alteration in the plasma or serum, for healthy corpuscles are caused to aggregate in a similar manner by mixing them with the diseased serum.

In some respects, nagana recalls the condition known as pernicious anaemia in man; and it may be noted that in 1897, I inoculated several rats with blood taken from patients suffering from this complaint. Cases are rare and I was not successful in trying from a case, which had not had arsenic administered therapeutically. At the same time several rats were injected with blood from patients suffering

from various kinds of leuchaemia. None of these animals showed any deviation from health or signs of parasites.

There is another blood change in nagana which on the whole is best seen in the rat when in an advanced stage of the disease. This change is manifested by a change of colour of the blood from the brilliant scarlet to a dull purplish or chocolate colour. The contrast between the healthy blood and the diseased blood is very striking when it is kept from coagulating by means of citrate, and can be recognised at a distance of some yards. In the clotted condition the phenomenon is likewise apparent, though hardly so marked. This dull coloured blood may be shaken with air, or allowed to stand for a week or more without developing the full red of normal oxyhaemoglobin. By the addition of the diseased blood to healthy blood the colour of the mixture is more or less dulled, so that the presence of healthy haemoglobin is not capable of causing a discharge of the abnormal colour. Examination with the spectroscope showed the bands of oxyhaemoglobin. Dr F. G. Hopkins kindly examined a specimen and considered that the oxyhaemoglobin bands did not appear quite normal. Dr Haldane has kindly drawn my attention to some observations[1] on the blood colour in poisoning with nitrobenzol derivatives. It appears that some species exhibit such changes whilst others do not do so ; thus nitrobenzol causes no blood colour change in mice, though it does in dogs and cats. With dinitrobenzol the blood of the rabbit becomes chocolate coloured ; this seems in part due to the presence of methaemoglobin but some other abnormal pigment is also probably at work.

Considerations such as these lead to the thought that though we now know much of the life-history and morphology of the parasites, this knowledge has taught us little, if indeed anything, concerning the disease itself. The same may be said of malaria in which morphology has not availed to advance our knowledge beyond diagnostic and preventive measures, the real nature of the disease and its symptoms yet remain to be unfolded. Another blood change, which may be mentioned as a matter requiring further elucidation, was noted in nagana-cachectic rabbits and consisted in the comparative resistance to haemolysis by the quillaia saponins. These saponins (sapotoxin and quillaiic acid) are capable of haemolysing red blood corpuscles in high degree, the extent varying in different species of animals. If 1 °/₀ dilutions of normal blood are treated with the glucosides it is found

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[1] Haldane, Makgill and Mavrogordato, *Journ. of Physiology*, xxi. Nitrobenzol and dinitrobenzol, p. 184.

that small additions of serum of normal blood have some sheltering or delaying action. Normal cat or dog serum has a greater delaying effect than normal rabbit serum, but it was found that the serum of rabbits in the highly cachectic condition of the later stages of nagana had a still greater effect. von Dungern attributes variation in this response to hæmolysis to the amount of lecithin substances in the case of normal animals; such may also be the cause in nagana.

Summary.

1. Some cases of remarkable resistance to nagana infection are recorded.

2. Although such birds as pigeons are unable to harbour the trypanosoma brucei, the kestrel is able to do so.

3. Attention is drawn to certain changes which are brought about by the trypanosome infection and the need for more precise chemical investigation of these hæmatozoal diseases.

Notes on blood parasites observed in Christmas Island (Straits Settlements) and in the Malay Peninsula.

Christmas Island possesses quite a number of peculiar species in its fauna, and it is regrettable that observations were not made before animals had been imported to this isolated station, as well as that my own notes are so incomplete.

Rats. There are three species of rat (1) the large *Mus nativitatis* had already become rare about the settlement at the time of my visits and I was unable to obtain either a freshly killed or a living specimen although a reward was offered.

(2) *Mus macleari.* A number of these rats was obtained and examined, of these nine were collected about the settlement; all were males and two of them had abundant trypanosomes in their blood. The trypanosome appeared morphologically like the *T. lewisi* of English rats.

Two other *M. macleari* were caught on the top of Phosphate Hill, some three or four miles from the settlement, one was a male the other a female and both were free from infection. Another was taken about half way up the hill and showed abundant trypanosomes in its blood;

the coat of this specimen was very infested with fleas[1], but there were none of the small ticks which were found on other specimens.

It is, perhaps, noteworthy that the spleen of this rat was very markedly enlarged, as also were the superficial lymphatic glands. This animal was a rover and possibly had acquired its infection from imported *Mus rattus*. The presence of more lesion than occurs usually with *T. lewisi* infections led to a working hypothesis that the annihilation of native rats by imported ship rats may be due to the introduction of trypanosomes, which, finding a " virgin soil " to work upon, cause fatal epidemics. Unfortunately all the later specimens that were examined proved to be free of infection.

Out of 12 specimens examined 3 (or 25 %) were affected with trypanosomiasis.

(3) *Mus rattus.* The specimens were identified at the British Museum, they varied much in colour from the so-called " grey " to black. The manager of the Phosphate Company, Captain Vincent, informed me that these rats were first introduced to the Island in December 1899 by the SS. *Hindustan* in a cargo of hay; they had multiplied to very great numbers at the time of my visit 1901–1902, but apparently they remained about the settlement. Altogether 13 of these rats were examined haematologically, and six of them were found to be harbouring a trypanosome of similar appearance to *T. lewisi.* In regard to this parasite, the presumption is, that it was introduced.

A species of biting fly, much like our *Stomoxys calcitrans,* was also very prevalent about the settlement and might spread infection.

Bats. Two specimens of the large *Pteropus natalis* were examined and both had infection with a small malaria-like parasite in their red corpuscles. Sporulation was not taking place at the time of examination. The coats of the bats were full of a louse-like parasite. Other means of spread of the infection is to be found in the mosquitos. I took three species on the Island, *Culex alis* (nov. sp. Theobald) being a new one, *C. fatigans* (only few specimens seen) and *Stegomyia scutellaris* which was very common and active during the day; the activity of the latter during the sleeping period of the bats would favour their attack.

Birds. The small ground pigeon, *Chalcophaps natalis,* was examined

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[1] *Loemopsylla nesiotes* sp. nov. (Jordan and Rothschild, *Parasitology*, vol. i. p. 1. 1908). The Hon. N. C. Rothschild has kindly informed me that all the specimens I obtained from *M. macleari* were of this peculiar species, so that an interchange of fleas from *M. rattus* is not proven.

once and found to be severely infested with a halteridium-like parasite, the specimen was taken near the settlement. Here again there is doubt whether the parasite existed as an original inhabitant, for a number of carrier pigeons had been introduced.

Carpophaga whartoni was not examined, and *Zosterops natalis* was examined but showed no blood parasite.

Of the three blood parasites, in rat, bat and pigeon, those of the rat and pigeon have probably been introduced, whilst that of the bat seems likely to have been an old standing native occurrence.

Whilst on the topic of animal infections it may be mentioned that I made a number of blood examinations on birds at Kuala Lumpur Federated Malay States, often with the kind help of Dr J. D. Gimlette. The ordinary pigeons showed heavy infection with *Halteridium*, so also did two pet specimens of a small green parrot. The sparrows and a small species of pie were all free of infection, though several of each were examined.

The observations on nagana were carried on with the aid of the Tsetse fly Committee of the Royal Society, and the other notes were made during the Beriberi expedition of the London School of Tropical Medicine.